Chemistry of Heterocyclic Compounds, Vol. 45, No. 12, 2009

SYNTHESIS AND CYTOTOXIC ACTIVITY OF DERIVATIVES OF 6*Z*-ACETYLMETHYLENE-PENICILLANIC ACID *tert*-BUTYL ESTER

M. Vorona[1]*, I. Potorochina[1], G. Veinberg[1], S. Belyakov[1], I. Shestakova[1], I. Kanepe[1], and E. Lukevics[1]†

The sulfoxide of 6Z-[2-(methoxyimino)propylidene]penicillanic acid tert-butyl ester and the sulfones of 6Z-[2-(hydroxyimino-, methoxyimino-, benzyloxyimino-, 2-bromo- and 4-bromobenzyloxyimino)-propylidene]penicillanic acid in the syn and anti forms have been synthesized by the condensation of the sulfoxide and sulfone of 6Z-acetylmethylenepenicillanic acid tert-butyl ester with hydroxylamine, methoxyamine, benzyloxyamine, 2-bromo- and 4-bromobenzyloxyamines. The syn and anti isomers of 3Z-(2-methoxyiminopropylidene)-4R-(benzothiazolyl-2-dithio)-2-oxoazetidinyl-1R-(2-propenyl)acetic acid tert-butyl ester were obtained by opening of the thiazolidine ring in 6Z-[2-(methoxyimino)propylidene]-1-oxopenicillanic acid tert-butyl ester with 2-mercaptobenzothiazole. The 3Z-(2-methoxyiminopropylidene)-4R-(methylsulfonyl)-2-oxoazetidinyl-1-(2-propylidene)acetic acid tert-butyl ester was synthesized by the interaction of 1,8-diazobicyclo[5.4.0]undec-7-ene and methyl iodide with 6Z-[2-(methoxyimino)propylidene]-1,1-dioxopenicillanic acid tert-butyl ester. A dependence of the cytotoxic effect in relation to cancer and normal cells in vitro on the structure of the substituent in position 6 and the syn and anti isomerism of the oxyimino group was established for the synthesized compounds.

Keywords: *tert*-butyl esters of 4*R*-(benzothiazolyl-2-dithio)-3*Z*-(2-methoxyiminopropylidene)-2-oxoazetidinyl-1*R*-(2-propenyl)acetic, 6*Z*-[2-(methoxyimino)propylidene]-1-oxopenicillanic, 4*R*-(methylsulfonyl)-3*Z*-(2-methoxyiminopropylidene)-2-oxoazetidinyl-1-(2-propylidene)acetic, 6*Z*-[2-(hydroxyimino)propylidene]-1,1-dioxopenicillanic, 6*Z*-[2-(methoxyimino)propylidene]-1,1-dioxopenicillanic, 6*Z*-[2-(benzyloxyimino)propylidene]-1,1-dioxopenicillanic, 6*Z*-[2-(2-bromobenzyloxyimino)propylidene]-1,1-dioxopenicillanic, 6*Z*-[2-(4-bromobenzyloxyimino)propylidene]-1,1-dioxopenicillanic acids, anticancer activity.

It was established by us previously that condensation of the acetylmethylene group in 7*Z*-acetylmethylene-3-methyl-1,1-dioxo-3-cephem-4-carboxylic acid *tert*-butyl ester with hydroxylamine or arylmethoxamines leads to the preparation of hydroxy- and arylmethoxyimino derivatives of cephem **1** in the *syn*

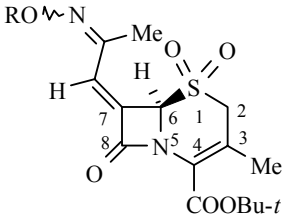
† Deceased.

* To whom correspondence should be addressed, e-mail: max@osi.lv, veinberg@osi.lv.

[1]Latvian Institute of Organic Chemistry, Riga LV-1006, Latvia.

Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 12, pp. 1897-1905, December, 2009. Original article submitted September 18, 2009.

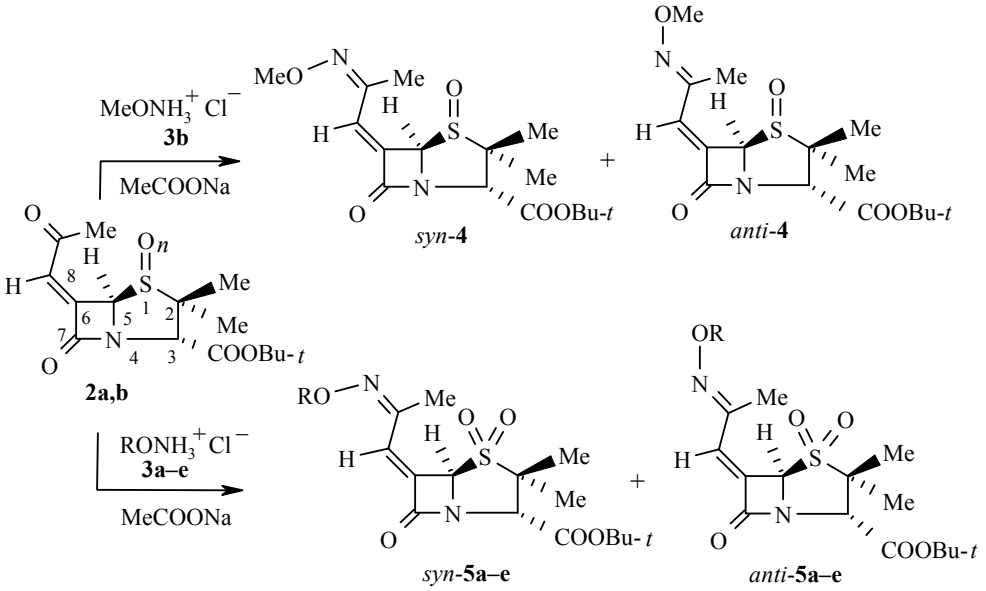
and *anti* isomeric forms, characterized by the presence of selective cytotoxic properties in relation to cancer cells [1]. In continuing this investigation it seemed expedient to synthesize and subject to cytotoxic screening similarly modified penams and also their derivatives with cleavage of the S(1)–C(2) bond.



1

R = H, $ArCH_2$, $PyCH_2$

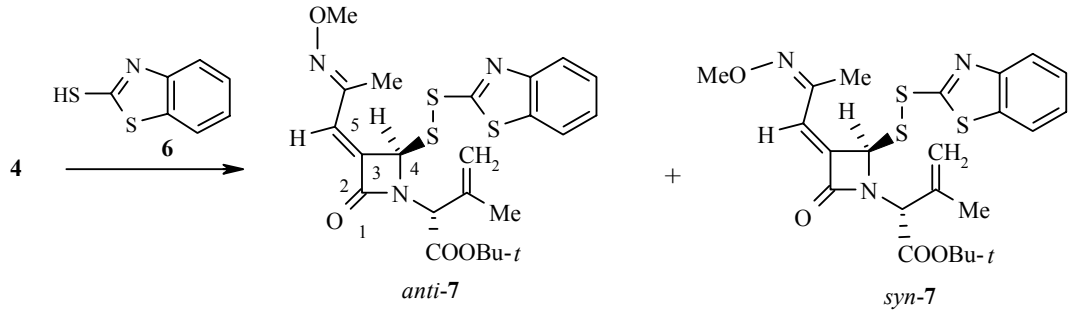
For this purpose the sulfoxide and sulfone of 6*Z*-acetylmethylenepenicillanic acid *tert*-butyl ester **2a,b**, synthesized by us previously in [2], were treated with the hydrochlorides of hydroxylamine **3a**, methoxyamine **3b**, and benzyloxyamines **3c-e**. These reactions were carried out at room temperature in methanol in the presence of sodium acetate. The condensation products **4** and **5a-e** were formed as mixtures of *syn* and *anti* isomers, in the case of **4** and **5a** they were separated into individual isomers with the aid of column chromatography.



3,5 a R = H, **b** R = Me, **c** R = $PhCH_2$, **d** R = 2-$BrC_6H_4CH_2$, **e** R = 4-$BrC_6H_4CH_2$;
2 a *n* = 1, **b** *n* = 2

The *syn* and *anti* configurations of the oxyimino fragments in compounds **4** and **5a-e** were identified with the aid of ^{1}H NMR spectroscopy based on the results of an analysis of the Overhauser effect for analogously modified cephalosporins [1]. According to this the spectra of the *syn* isomers of **4, 5a-e** are characterized by a low field displacement of the signal of the H-8 proton in comparison with the analogous shift in the *anti* isomers of **4, 5a-e**.

The oxyiminopropylidene structure of the substituent in position **6** of the obtained compounds does not prevent the execution of reactions directed at cleavage of the S(1)–C(2) bond of the penam nucleus in them. The sulfoxide of 6*Z*-[2-(methoxyimino)propylidene]penicillanic acid *tert*-butyl ester (**4**) was converted with the aid of 2-mercaptobenzothiazole (**6**) into 3*Z*-(2-methoxyiminopropylidene)-4*R*-(benzothiazolyl-2-dithio)-2-oxo-azetidinyl-1*R*-(2-propenyl)acetic acid *tert*-butyl ester (**7**) as *syn* and *anti* isomers.



The signals of the H-5 protons in the ^{1}H NMR spectra of *anti*- and *syn*-**7**, unlike those of the previously mentioned compounds **4, 5a-e**, are characterized by closely similar values of chemical shifts. In this case, by analogy with the penams *anti*-**4** and *anti*-**5**, the *anti* configuration of the methoxyimino group is proposed for the less polar *anti*-**7** stereoisomer.

The structural identification of substance *anti*-**7** by X-ray structural analysis (Fig. 1) confirmed the correctness of this proposal. According to the X-ray structural analysis both heterocyclic systems of the molecule are planar within the limits of error. Valence angles C(2)–S(10)–S(11) and S(10)–S(11)–C(12) at the disulfide bridge were equal to 101.8(6) and 103.3(5)° respectively. The torsion angle C(2)–S(10)–S(11)–C(12) was 100.6(5)°. The bond length C(15)–C(28) of 1.33(2) Å corresponds to the length of an isolated double bond. All the intermolecular contacts in the crystal were effected at distances no less than the sum of the van der Waals radii of the contacting atoms.

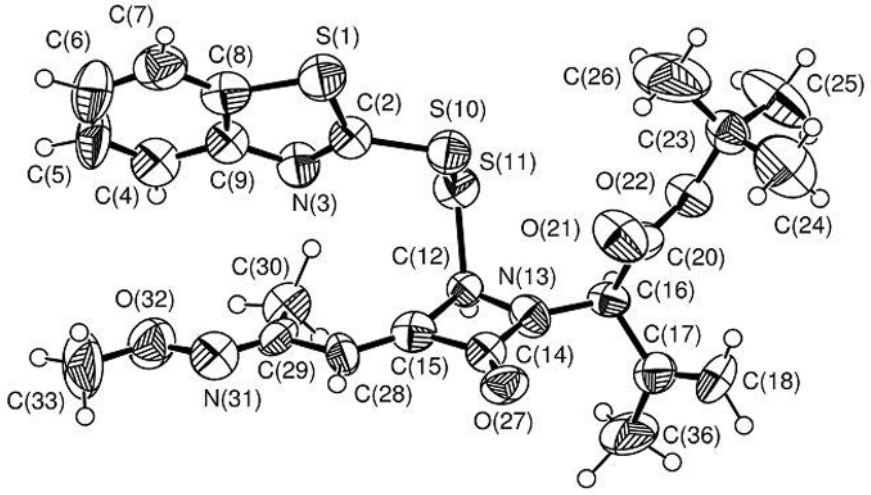
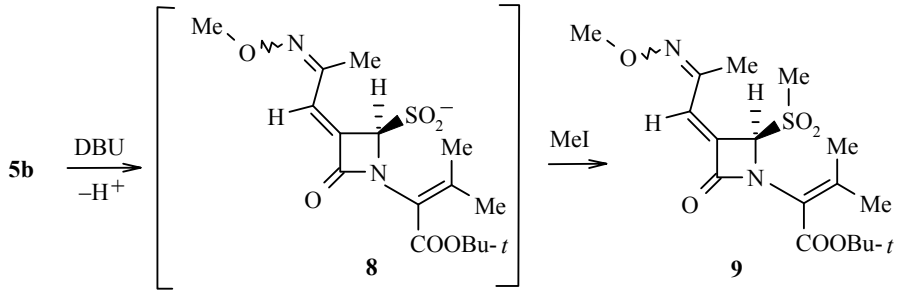


Fig. 1. Spatial model of the *anti*-**7** molecule with designation of atoms and with ellipses for thermal vibrations.



DBU = 1,8-diazobicyclo[5.4.0]undec-7-ene

TABLE 1. Biological Properties of Derivatives of 6*Z*-Acetylmethylenepenicillanic Acid *tert*-Butyl Ester*

Compound	LC_{50}, μg/ml							LD_{50}, μg/kg
	HT-1080			MG-22A			3T3	
	CV	MTT	TG_{100}	CV	MTT	TG_{100}	NR	
anti-**4**	2.3	3	350	1	1.5	350	10	321
syn-**4**	2.7	3	300	2.6	2.5	950	10	321
anti-**5a**	3	3	450	3	2	200	817	2151
syn-**5a**	3	2	140	2	2	140	17	394
anti/syn-**5b**	0.8	0.1	100	0.8	0.4	75	2.5	186
anti/syn-**5c**	2	2	300	2	2	300	5	269
*anti/syn***5d**	3	3	300	2	2	250	4	264
*anti/syn***5e**	2	2	300	2	2	350	6	316
anti/syn-**9**	4.1	8.6	167	10.2	18.2	300	77	812

*LC_{50} is the concentration providing death of 50% cells; CV, staining with Crystal Violet; MTT, staining with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; TG_{100}, specific $NO^{\bullet}$ generating activity of the compound; NR, staining with Neutral Red; LD_{50}, calculated value of the expected toxicity.

Deprotonation of the C(3) atom in 6*Z*-(2-methoxyiminopropylidene)penicillanic acid *tert*-butyl ester sulfone (**5b**) with 1,8-diazobicyclo[5.4.0]undec-7-ene leads to rupture of the S(1)-C(2) bond and the formation of the sulfinate anion **8**, by the alkylation of which with methyl iodide a mixture is synthesized of the *syn* and *anti* isomers of 3*Z*-(2-methoxyiminopropylidene)-4*R*-(methylsulfonyl)-2-oxoazetidinyl-1-(2-propylidene)acetic acid *tert*-butyl ester (**9**).

Biological screening of the synthesized substances *in vitro* comprised determination of their cytotoxic properties in relation to monolayers of cancer cell lines HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) in comparison with normal 3T3 cells (mouse embryonic fibroblasts). Staining of 3T3 fibroblasts with Neutral Red enabled calculation, with the aid of a special equation without conducting experiments *in vivo,* of an expected value of the LD_{50} for the test compounds [3].

The data of Table 1 indicate selective cytotoxicity in relation to cancer cells for the *anti* isomer **5a** of 6*Z*-[2-(hydroxyimino)propylidene]-1,1-dioxopenicillanic acid *tert*-butyl ester compared with the *syn* isomer of **5a**. This is indicated by the more than sixfold difference in estimated and expected values of their LD_{50}. O-Alkylation of the hydroxyimino group, accompanying the values of LD_{50} for compounds **5b-e**, is characterized by a weakening of the selectivity of the cytotoxic effect. On the other hand, opening of the thiazolidine ring in sulfone **5b,** leading to the monocyclic β-lactam **9,** assisted strengthening of this factor.

EXPERIMENTAL

The ^1H NMR spectra were recorded on a Varian Mercury 200 (200 MHz) instrument in $CDCl_3$ (compounds **4, 5a-e**, and **9**) and in DMSO-d_6 (compound **7**), internal standard was HMDS (δ 0.055 ppm). Elemental analysis was carried out on a Carlo Erba 1108 analyzer. A check on the progress of reactions was effected by TLC on Merck Kieselgel plates with visualization in UV light. Silica gel of type Merck Kieselgel (0.060-0.200 mm) was used for preparative column chromatography. Reagents and materials from Acros, Aldrich were used in experiments.

The optical density in the biological tests, carried out in 96-hole plates, was determined with a Tetretek Multiscan MCC/340 horizontal spectrophotometer.

6*Z*-[2-*anti*-(Methoxyimino)propylidene]-1-oxopenicillanic Acid *tert*-Butyl Ester (*anti*-4) and 6*Z*-[2-*syn*-(Methoxyimino)propylidene]-1-oxopenicillanic Acid *tert*-Butyl Ester (*syn*-4). Methoxyamine hydrochloride (37 mg, 0.44 mmol,) and sodium acetate (36 mg, 0.44 mmol) were added to a solution of 6*Z*-acetylmethylene-1-oxopenicillanic acid *tert*-butyl ester (**2a**) (100 mg, 0.31 mmol) in methanol (5 ml). The reaction mixture was stirred for 24 h at room temperature and evaporated at reduced pressure. The residue was fractionated on a chromatographic column of silica gel. Yield was 76 mg (69%). Found, %: C 54.10; H 6.86; N 7.91. $C_{16}H_{24}N_2O_5S$. Calculated, %: C 53.92; H 6.79; N 7.86.

From fractions with R_f 0.29 (eluent ethyl acetate–hexane, 1:1) *anti*-**4** was obtained. ^{1}H NMR spectrum, δ, ppm: 1.29 (3H, s, CH_3); 1.51 (9H, s, C_4H_9); 1.67 (3H, s, CH_3); 2.00 (3H, s, $C(CH_3)$=N); 4.00 (3H, s, OCH_3); 4.53 (1H, s, H-3); 5.56 (1H, br. s, H-5); 6.73 (1H, br. s, =C<u>H</u>C(CH_3)=N).

From fractions with R_f 0.17 (eluent ethyl acetate–hexane, 1:1) *syn*-**4** was obtained. ^{1}H NMR spectrum, δ, ppm: 1.30 (3H, s, CH_3); 1.52 (9H, s, C_4H_9); 1.70 (3H, s, CH_3); 2.12 (3H, s, CH_3C=N); 3.93 (3H, s, OCH_3); 4.57 (1H, s, H-3); 5.58 (1H, br. s, H-5); 7.39 (1H, br. s, =C<u>H</u>C(CH_3)=N).

Preparation of *tert*-Butyl Esters of 6*Z*-[2-(Hydroxyimino)propylidene]-1,1-dioxopenicillanic, 6*Z*-[2-(Methoxyimino)propylidene]-1,1-dioxopenicillanic, and 6*Z*-[2-Arylmethoxyimino)propylidene]-1,1-dioxopenicillanic Acids 5a-e (General Method). Oxyamine hydrochloride **3a-e** (1.92 mmol) and sodium acetate (157 mg, 1.92 mmol) were added to a solution of 6*Z*-acetylmethylene-1,1-dioxopenicillanic acid *tert*-butyl ester (**2b**) (344 mg, 1.00 mmol) in methanol (10 ml). The reaction mixture was stirred for 24 h at room temperature and evaporated under reduced pressure. The residue was fractionated on a chromatographic column of silica gel and penams **5a-e** were obtained.

***tert*-Butyl Esters of 6*Z*-[2-*anti*-(Hydroxyimino)propylidene]-1,1-dioxopenicillanic Acid (*anti*-5a) and 6*Z*-[2-*syn*-(Hydroxyimino)propylidene]-1,1-dioxopenicillanic Acids (*syn*-5a)** were obtained by the typical method using hydroxylamine hydrochloride. Yield was 73%. Found, %: C 50.41; H 6.22; N 7.78. $C_{15}H_{22}N_2O_6S$. Calculated, %: C 50.27; H 6.19; N 7.82.

From the fraction with R_f 0.40 (eluent ethyl acetate–hexane, 1:2) *anti*-**5a** was obtained. ^{1}H NMR spectrum, δ, ppm: 1.45 (3H, s, CH_3); 1.52 (9H, s, C_4H_9); 1.58 (3H, s, CH_3); 2.06 (3H, s, $C(CH_3)$=N); 4.32 (1H, s, H-3); 5.32 (1H, s, H-5); 6.88 (1H, s, =C<u>H</u>C(CH_3)=N); 9.42 (1H, br. s, OH).

From the fraction with R_f 0.31 (eluent ethyl acetate–hexane, 1:2) *syn*-**5a** was obtained. ^{1}H NMR spectrum, δ, ppm: 1.46 (3H, s, CH_3); 1.52 (9H, s, C_4H_9); 1.59 (3H, s, CH_3); 2.12 (3H, s, $C(CH_3)$=N); 4.37 (1H, s, H-3); 5.30 (1H, s, H-5); 7.47 (1H, s, =C<u>H</u>C(CH_3)=N); 8.59 (1H, br. s, OH).

Mixture of *tert*-Butyl Esters of 6*Z*-[2-*anti*-(Methoxyimino)propylidene]-1.1-dioxopenicillanic Acid (*anti*-5b) and 6*Z*-[2-*syn*-(Methoxyimino)propylidene]-1,1-dioxopenicillanic Acid (*syn*-5b) (3:2) was obtained by the typical method with methoxyamine hydrochloride. Yield was 79%. Found,%: C 51.81; H 6.66; N 7.57. $C_{16}H_{24}N_2O_6S$. Calculated, %: C 51.60; H 6.50; N 7.52. From the fraction with R_f 0.49 (eluent ethyl acetate–hexane, 1:2) a mixture of the *anti* and *syn* isomers of **5b** was obtained.

^{1}H NMR spectrum, δ, ppm: *anti*-**5b** – 1.46 (3H, s, CH_3); 1.52 (9H, s, C_4H_9); 1.58 (3H, s, CH_3); 2.00 (3H, s, $C(CH_3)$=N); 4.04 (3H, s, OCH_3); 4.32 (1H, s, H-3); 5.28 (1H, br. s, H-5); 6.84 (1H, br. s, =C<u>H</u>C(CH_3)=N); *syn*-**5b** – 1.46 (3H, s, CH_3); 1.52 (9H, s, C_4H_9); 1.58 (3H, s, CH_3); 2.11 (3H, s, $C(CH_3)$=N); 3.95 (3H, s, OCH_3); 4.36 (1H, s, H-3); 5.28 (1H, br. s, H-5); 7.48 (1H, br. s, =C<u>H</u>C(CH_3)=N).

Mixture of *tert*-Butyl Esters of 6*Z*-[2-*anti*-(Benzyloxyimino)propylidene]-1,1-dioxopenicillanic Acid (*anti*-5c) and 6*Z*-[2-*syn*-(Benzyloxyimino)propylidene]-1,1-dioxopenicillanic acid (*syn*-5c) (1:1) was obtained by the typical method using benzyloxyamine hydrochloride. Yield was 56%. Found, %: C 59.11; H 6.36; N 6.29. $C_{22}H_{28}N_2O_6S$. Calculated, %: C 58.91; H 6.29; N 6.25. A mixture of the *anti* and *syn* isomers of **5c** was obtained from the fraction with R_f 0.46 (eluent ethyl acetate–hexane, 1:3).

^{1}H NMR spectrum, δ, ppm: *anti*-**5c** – 1.44 (3H, s, CH_3); 1.51 (9H, s, C_4H_9); 1.58 (3H, s, CH_3); 2.04 (3H, s, $C(CH_3)$=N); 4.31 (1H, s, H-3); 5.24 (1H, br. s, H-5); 5.30 (2H, s, $C\underline{H}_2C_6H_5$); 6.82 (1H, br. s, =$C\underline{H}C(CH_3)$=N); 7.31-7.40 (5H, m, C_6H_5); *syn*-**5c** – 1.44 (3H, s, CH_3); 1.51 (9H, s, C_4H_9); 1.58 (3H, s, CH_3); 2.11 (3H, s, $C(CH_3)$=N); 4.35 (1H, s, H-3); 5.19 (2H, s, $C\underline{H}_2C_6H_5$); 5.24 (1H, br. s, H-5); 7.31-7.40 (5H, m, C_6H_5); 7.55 (1H, br. s, =$C\underline{H}C(CH_3)$=N).

Mixture of *tert*-Butyl Esters of 6*Z*-[2-*anti*-(2-Bromobenzyloxyimino)propylidene]-1,1-dioxopenicillanic Acid (*anti*-5d) and 6*Z*-[2-*syn*-(2-Bromobenzyloxyimino)propylidene]-1,1-dioxopenicillanic Acid (s*yn*-5d) (2:3) was obtained by the typical method using 2-bromobenzyloxyamine. Yield was 65%. Found, %: C 50.26; H 5.21; N 5.37. $C_{22}H_{27}BrN_2O_6S$. Calculated, %: C 50.10; H 5.16; N 5.31. A mixture of the *anti* and *syn* isomers of **5d** was obtained from fractions with R_f 0.51 (eluent ethyl acetate–hexane, 1:3).

^{1}H NMR spectrum, δ, ppm: *anti*-**5d** – 1.46 (3H, s, CH_3); 1.51 (9H, s, C_4H_9); 1.59 (3H, s, CH_3); 2.07 (3H, s, $C(CH_3)$=N); 4.32 (1H, s, H-3); 5.28 (1H, br. s, H-5); 5.39 (2H, s, $C\underline{H}_2C_6H_5$); 6.83 (1H, br. s, =$C\underline{H}C(CH_3)$=N); 7.11-7.45 (3H, m, H-4,5,6 C_6H_4); 7.52-7.59 (1H, m, H-3 C_6H_4); *syn*-**5d** – 1.46 (3H, s, CH_3); 1.51 (9H, s, C_4H_9); 1.59 (3H, s, CH_3); 2.12 (3H, s, $C(CH_3)$=N); 4.36 (1H, s, H-3); 5.28 (3H, br. s, H-5, $C\underline{H}_2C_6H_4$); 7.11-7.45 (3H, m, H-4,5,6 C_6H_4); 7.52-7.59 (1H, m, H-3 C_6H_4); 7.60 (1H, br. s, =$C\underline{H}C(CH_3)$=N).

Mixture of *tert*-Butyl Esters of 6*Z*-[2-*anti*-(4-Bromobenzyloxyimino)propylidene]-1,1-dioxopenicillanic Acid (*anti*-5e) and 6*Z*-[2-*syn*-(4-Bromobenzyloxyimino)propylidene]-1,1-dioxopenicillanic Acid (*syn*-5e) (3:2) was obtained by the typical method using 4-bromobenzyloxyamine. Yield was 62%. Found, %: C 50.28; H 5.25; N 5.33. $C_{22}H_{27}BrN_2O_6S$. Calculated, %: C 50.10; H 5.16; N 5.31. A mixture of the *anti* and *syn* isomers of **5e** was obtained from fractions with R_f 0.46 (eluent ethyl acetate–hexane, 1:3).

^{1}H NMR spectrum, δ, ppm: *anti*-**5e** – 1.45 (3H, s, CH_3); 1.51 (9H, s, C_4H_9); 1.58 (3H, s, CH_3); 2,03 (3H, s, $C(CH_3)$=N); 4.31 (1H, s, H-3); 5.20-5.27 (3H, m, H-5, CH_2Ph); 6.80 (1H, d, ^4J = 1.5, =$C\underline{H}C(CH_3)$=N); 7.23 (2H, d, ^3J = 8.8, H-2,6 C_6H_4); 7.48 (2H, d, ^3J = 8.8, H-3,5 C_6H_4); *syn*-**5e** – 1.45 (3H, s, CH_3); 1.51 (9H, s, C_4H_9); 1.58 (3H, s, CH_3); 2.10 (3H, s, $C(CH_3)$=N); 4.35 (1H, s, H-3); 5.12 (1H, s, H-5); 5.23 (2H, br. s, $C\underline{H}_2C_6H_4$); 7.23 (2H, d, ^3J = 8.8, H-2,6 C_6H_4); 7.42-7.53 (3H, m, H-3,5 C_6H_4,=$C\underline{H}C(CH_3)$=N).

***tert*-Butyl Esters of 3*Z*-(2-*anti*-Methoxyiminopropylidene-4*R*-(benzothiazolyl-2-dithio)-2-oxoazetidinyl-1*R*-(2-propenyl)acetic Acid (*anti*-7) and 3*Z*-(2-*syn*-Methoxyiminopropylidene)-4*R*-(benzothiazolyl-2-dithio)-2-oxoazetidinyl-1*R*-(2-propenyl)acetic Acid (*syn*-7).** 2-Mercaptobenzothiazole (430 mg, 2.57 mmol) and 3 Å molecular sieves (3 g) were added to a solution of 6*Z*-[2-(methoxyimino)propylidene]-1-oxopenicillanic acid *tert*-butyl ester (**4**) (800 mg, 2.25 mmol) in toluene (30 ml). The reaction mixture was boiled for 3 h, cooled, filtered, and evaporated at reduced pressure. The residue was fractionated on a chromatographic column of silica gel.

Crystals of *anti*-**7** precipitated on evaporation of fractions with R_f 0.37 (eluent ethyl acetate–hexane, 1:5). Yield was 182 mg (16%); mp 127°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.56 (9H, s, C_4H_9); 1.92 (3H, s, CH_3); 1.97 (3H, s, $C(CH_3)$=N); 3.80 (3H, s, OCH_3); 4.86 (1H, s, CHCOO); 5.11 (2H, d, ^2J = 13.5, C=CH_2); 5.94 (1H, d, ^4J = 1.4, H-4); 6.37 (1H, d, ^4J = 1.4, =$C\underline{H}C(CH_3)$=N); 7.26-7.52 (2H, m, H-5,6 C_6H_4); 7.70-7.97 (2H, m, H-4,7 C_6H_4). Found, %: C 54.41; H 5.33; N 8.16. $C_{23}H_{27}N_3O_4S_3$. Calculated, %: C 54.63; H 5.38; N 8.31.

Crystals of *syn*-**7** precipitated on evaporation of fractions with R_f 0.24 (eluent ethyl acetate–hexane, 1:5). Yield was 80 mg (7%); mp 131°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.45 (9H, s, C_4H_9); 1.93 (3H, s, CH_3); 2.22 (3H, s, $C(CH_3)$=N); 3.77 (3H, s, OCH_3); 4.86 (1H, s, CHCOO); 5.14 (2H, d, ^2J = 17, C=CH_2); 5.82 (1H, s, H-4); 6.34 (1H, s, =$C\underline{H}C(CH_3)$=N); 7.24-7.52 (2H, m, H-5,6 C_6H_4); 7.73-7.97 (2H, m, H-4,7 C_6H_4). Found, %: C 54.58; H 5.45; N 8.33. $C_{23}H_{27}N_3O_4S_3$. Calculated, %: C 54.63; H 5.38; N 8.31.

Mixture of *tert*-Butyl Esters of 3*Z*-(2-*anti*-Methoxyiminopropylidene)-4*R*-(methylsulfonyl)-2-oxoazetidinyl-1-(2-propylidene)acetic Acid (*anti*-9) and 3*Z*-(2-*syn*-Methoxyiminopropylidene)-4*R*-(methylsulfonyl)-2-oxoazetidinyl-1-(2-propylidene)acetic Acid (*syn*-9). 1,8-Diazobicyclo[5.4.0]undec-7-ene (45 μlite, 0.30 mmol) and, after 5 min, methyl iodide (19 μliter, 0.30 mmol) were added to a solution of 6*Z*-[2-(methoxyimino)propylidene]-1,1-dioxopenicillanic acid *tert*-butyl ester (**5b**) (100 mg, 0.27 mmol) in dichloro-

methane (7 ml) at 0°C. The mixture was stirred for 1 h at room temperature, diluted with dichloromethane (15 ml), washed with 0.5 N HCl (10 ml), 5% NaCl solution (2×10 ml), dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under reduced pressure. The residue was fractionated on a chromatographic column of silica gel. A mixture of *anti* and *syn* isomers of **9** was obtained from fractions with R_f 0.43 (eluent ethyl acetate–hexane, 1:1). Yield was 31 mg (34%). Found, %: C 52.97; H 6.81; N 7.26. $C_{17}H_{26}N_2O_6S$. Calculated, %: C 52.83; H 6.78; N 7.25

^{1}H NMR spectrum, δ, ppm (*J*, Hz): *anti*-**9** – 1.51 (9H, s, C_4H_9); 2.07 (3H, s, =C(CH_3)C$\underline{H}_3$); 2.19 (3H, s, C(CH_3)=N); 2.23 (3H, s, =C(CH_3)C$\underline{H}_3$); 2.85 (3H, s, SO_2CH_3); 4.00 (3H, s, OCH_3); 5.65 (1H, d, ^4J = 1.4, H-4); 7.24 (1H, d, ^4J = 1.4, =C$\underline{H}$C(CH_3)=N); *syn*-**9** – 1.51 (9H, s, C_4H_9); 2.06 (3H, s, =C(CH_3)C$\underline{H}_3$); 2.23 (6H, s, C(CH_3)=N, =C(CH_3)C$\underline{H}_3$); 2.85 (3H, s, SO_2CH_3); 3.93 (3H, s, OCH_3); 5.81 (1H, d, ^4J = 1.4, H-4); 6.82 (1H, d, ^4J = 1.4, =C$\underline{H}$C(CH_3)=N).

X-ray Structural Analysis. A diffraction picture was obtained from a monocrystal of compound *anti*-**7** of size 0.03×0.04×0.21 mm on a Nonius KappaCCD automatic diffractometer up to $2\theta_{max} = 45^o$ (l_{Mo} = 0.71073 Å). Monocrystals of *anti*-**7** were grown from ethyl acetate–hexane: a = 5.612(3), b = 12.451(5), c = 18.672(9) Å, β = 96.66(2)°; V = 1295.9(9) Å^3, $F(000)$ = 532, μ = 0.319 mm^{-1}, d_{calc} = 1.296 g.cm^{-3}, Z = 2, space group $P2_1$. Of 2214 independent reflections 1509 with $I > 2\sigma(I)$ were used in the calculations. The structure was solved by the procedure of [4]. Refinement was carried out by the least squares method in a full-matrix anisotropic approximation with the SHELXL97 set of programs [5]. The final value of the divergence factor R = 0.0964. Complete crystallographic information for *anti*-**7** has been deposited in the Cambridge structural data bank (CCDC 746815).

Determination of Cytotoxic Activity *in vitro*. The cytotoxic properties of the synthesized substances in relation to cultures of monolayers of cancer and normal cells at $c = (2\text{-}5).10^4$ cells/ml of HT-1080, MG-22A, and 3T3 lines were determined on 96-hole plastic panels using dyestuffs CV, MTT, and NR in accordance with the procedures of [6].

Generation of NO$^\bullet$ Radicals by Cells. Determination of the concentration of nitric oxide radicals in the cell medium according to Griess [7] was carried out in 96-hole plastic panels. The obtained concentrations (nmol) of NO$^\bullet$ radicals in the culture medium with surviving cells, after incubation for 72 h in the presence of the test substance at a concn. of 50 μg/ml in a hole of volume 200 μliter, were used to calculate values of the specific NO$^\bullet$ generating activity of compounds (TG_{100}).

$$TG_{100} = G\ 100/\ C\ (\text{nmol}/\mu\text{l}),$$

where G is the NO$^\bullet$ concentration (nmol) in a culture medium volume of 200 μliter with surviving cells; C is the percentage of surviving cells determined by staining with CV.

REFERENCES

1. I. Potorochina, M. Vorona, G. Veinberg, I. Shestakova, I. Kanepe, M. Petrova, E. Liepinsh, and E. Lukevics, *Khim. Geterotsikl. Soedin.*, 284 (2009). [*Chem. Heterocycl. Comp.*, **45**, 228 (2009)].
2. G. Veinberg, I. Shestakova, M. Vorona, I. Kanepe, and E. Lukevics, *Bioorg. Med. Chem. Lett.*, **14**, 1007 (2004).
3. *Guidance Document on Using in vitro Data to Estimate in vivo Starting Doses for Acute Toxicity*, National Institutes of Health, (2001). US Dept. of Health and Human Services.
4. A. Altomare, M. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Guagliardi. A. Moliterni, and R. Spagna, *J. Appl. Crystallogr.*, **32**, 115 (1999).
5. G. M. Sheldrick, *Acta Crystallogr.,* **A64**, 112 (2008).
6. P. J. Freshney, in *Culture of Animal Cells (Manual of Basic Technique)*, Wiley-Liss, New York (1994), p. 296.
7. D. J. Fast, R. C. Lynch, and R. W. Leu, *J. Leukocyt. Biol.*, **52**, 255 (1992).